# Isolation and characterization of a 7 S RNP particle from mature *Xenopus laevis* oocytes

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Mature oocytes of *Xenopus laevis* contain a 7 S RNP particle consisting of two components, ribosomal 5 S RNA and a protein of $M_r$ ~45000. The structure of the free 5 S rRNA and the 7 S RNP complex has been studied by diethylpyrocarbonate modification of adenines. $A_{74}$, $A_{77}$, $A_{90}$, $A_{100}$, $A_{101}$ and $A_{103}$ of the 5 S rRNA are protected upon association of the protein.

Xenopus laevis *7 S RNP* *5 S rRNA* *Chemical modification* *Protein–RNA interaction*

## 1. INTRODUCTION

In the early stages of *Xenopus laevis* oogenesis 5 S ribosomal RNA has been shown to be stored in association with a protein of $M_r$ ~45000 forming a 7 S ribonucleoprotein (RNP) particle [1] or with tRNA and two other proteins in a 42 S RNP particle [2,3]. The 7 S protein interacts specifically with an intragenic control region in the 5 S rRNA genes [4–6] functioning as a positive transcription factor [4,7]. The interaction of the 7 S protein and the *Xenopus* 5 S rRNA gene has been described in detail [4–6].

Here, we report on the isolation of 7 S RNP particles from mature *Xenopus laevis* oocytes. In order to characterize the structure of the RNA component from this particle we have carried out chemical modification studies of adenines in the free 5 S rRNA and in the 7 S RNP particle, using diethylpyrocarbonate [8]. This reagent is thought to react preferentially at the N-7 position of unstacked adenines under physiological conditions.

## 2. MATERIALS AND METHODS

### 2.1. *Isolation of the 7 S RNP particle*

A two step procedure for the isolation of 7 S RNP particles from mature *Xenopus laevis* oocytes (a gift from Dr B. Appel) was used, which essentially corresponds to the method of B. Picard et al. [3]. After homogenization in 20 mM Tris–HCl (pH 7.5)/70 mM KCl/10 mM $MgCl_2$/3 mM DTT, the homogenate was centrifuged at 13000 × *g* for 10 min. The clear supernatant was then layered on top of a 10–40% sucrose gradient in homogenization buffer and centrifuged at 83000 × *g* (Beckman, SW 28) for 18 h. Aliquots of the gradient fractions were screened for 7 S RNP particles by analytical gel electrophoresis under non-denaturing conditions [3]. 7 S RNP containing fractions were dialyzed against 50 mM Tris–HCl (pH 6.8)/5 mM $MgCl_2$ (buffer W) and loaded on a Whatman DE 52 anion exchange column (1.5 × 12 cm); particles were eluted with a 0–0.6 M NaCl gradient [3] prepared in the same buffer (fig.1). All the operations were carried out at 0–4°C. Free 5 S rRNA was isolated from RNP particles by phenol extraction [3].

### 2.2. *Adenine modification*

0.5 $A_{260}$ units of 3′-end labelled [9] *Xenopus laevis* 5 S rRNA were incubated with 25 μl diethylpyrocarbonate in 500 μl 50 mM Na-cacodylate/50 mM KCl/10 mM $MgCl_2$/pH 7.5 for 30 min at 30°C. The reaction was terminated by

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the addition of 125 $\mu$l 1.5 M sodium acetate and the RNA was ethanol precipitated. 7 S RNP particles were modified under the same conditions, but the reaction was terminated by Sephadex G-100 gel filtration in buffer W and 7 S RNP particles separated from free 5 S rRNA and free proteins by ion-exchange chromatography as above. The RNA was phenol extracted, 3′-end labelled [9] and purified by polyacrylamide gel electrophoresis as was the modified free 5 S rRNA. The oligonucleotide fragments generated by aniline treatment of the modified RNAs were identified as described [8,10].

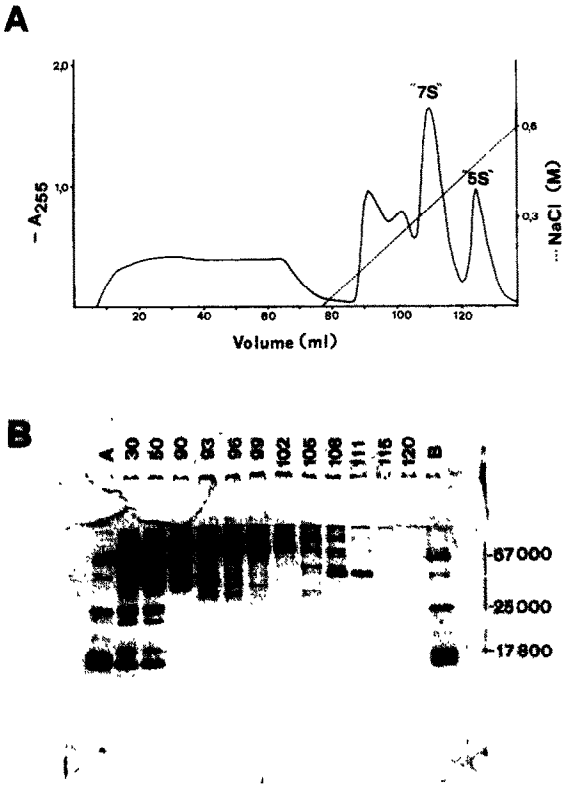


Fig.1. (A) Elution profile of a crude 7 S RNP fraction from sucrose gradient centrifugation applied to an anion exchange column (Whatman DE 52) and eluted with a 0–0.6 M NaCl gradient. The peaks containing the 7 S RNP particle and 5 S rRNA are indicated. (B) Examination of aliquots from fractions of the anion exchange column as indicated in fig.1A on a 15% polyacrylamide–SDS gel stained for protein. $M_r$ markers were applied to lanes A and B.

## 3. RESULTS

The experimental results of the two step isolation procedure for 7 S RNP particles from mature

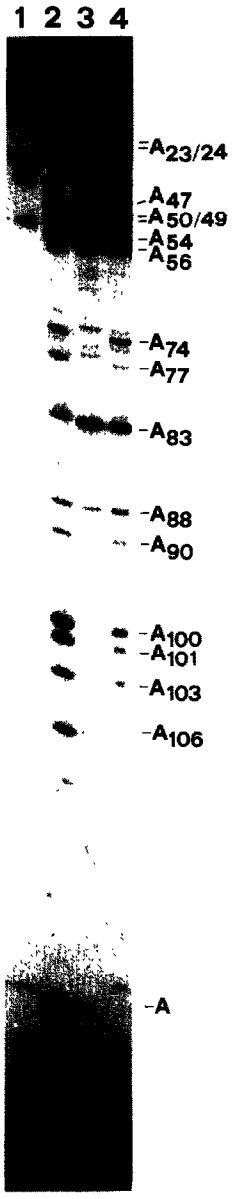


Fig.2. Identification of diethylpyrocarbonate modified adenines in the free 5 S rRNA (lane 4) and in the 7 S RNP particle (lane 3). Total modification of adenines (lane 2) was performed at 90°C under sequencing conditions [18] and aniline-treated unmodified 5 S rRNA is shown in lane 1. Electrophoresis was carried out at 1.5 kV on a 12% polyacrylamide gel (60 × 20 × 0.05 cm) under denaturing conditions for 6 h.

*Xenopus laevis* oocytes are shown in fig.1. About 1% of the total protein in the clear cellular homogenate was eluted from the ion exchange column (fig.1A) as 7 S RNP-associated protein. Judging from the protein pattern of the stained SDS gel (fig.1B) the purity of this protein was >90%. We saw no difference in the composition of the phenol extracted RNA from the 7 S peak compared to the fraction eluting as the ribosomal RNA 5 S RNA peak (not shown). Both fractions contained slightly fragmentated 5 S rRNA and no detectable tRNA.

Using the diethylpyrocarbonate reaction we were able to modify the adenines in position 23, 24, 49, 50, 54, 56, 74, 77, 83, 88, 90, 100, 101 and 103 in the free 5 S rRNA. The most extensive modification occurred at $A_{83}$. In the 7 S RNP particle we modified the adenines in position 23, 24, 49, 50, 54, 56, 83, 88 and 90.

The reactivity of $A_{90}$ was reduced in comparison to the free 5 S rRNA. The identification of the oligonucleotides, generated by aniline treatment of the modified RNAs, by sequencing gel analysis is shown in fig.2 and the results are summarized in fig.3.

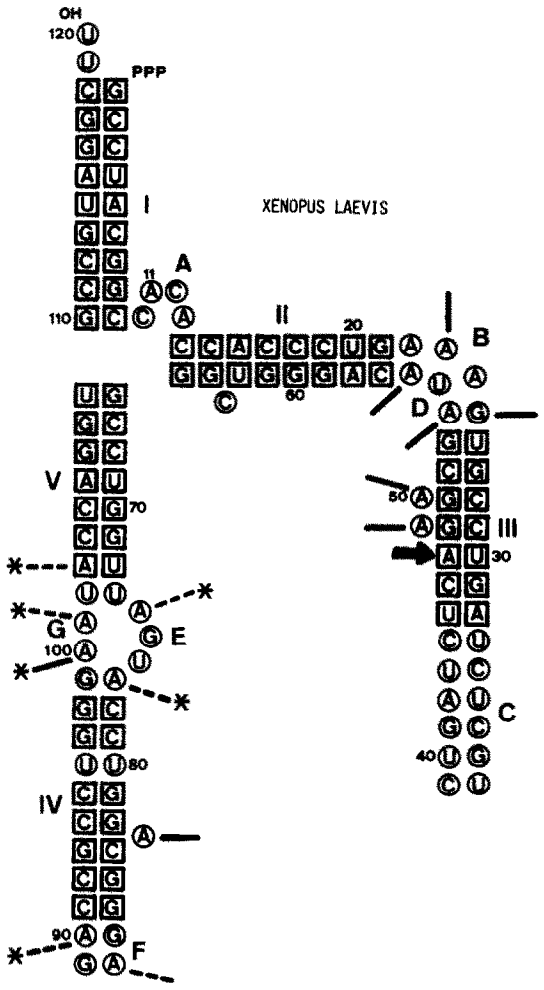


Fig.3. Secondary structural model of *Xenopus laevis* (oocyte) 5 S rRNA (sequence from [19]), according to the general eukaryotic 5 S rRNA structure [14]. Lines indicate modification sites in the free 5 S rRNA and stars indicate protection from modification in the 7 S RNP particle. $A_{47}$, which is accessible only upon binding of the protein, is marked by an arrow.

## 4. DISCUSSION

Clear evidence is presented for the existence of 7 S RNP particles in mature oocytes of *Xenopus laevis*. The RNA component is identical to the transcript of oocyte 5 S rRNA genes, which agrees with reports about the persistence of non-ribosome bound 5 S rRNA in fully grown oocytes of *Xenopus laevis* [11], sedimenting at about 7 S. The 42 S particle observed in earlier stages of oocyte development was not detected. In addition the quantity and ratio of 7 S RNP and free 5 S rRNA from the ion-exchange chromatography rule out the possibility that the 7 S particles isolated originate from those few oocytes in the mature ovary, which are in an early stage of oogenesis.

However, conflicting results have been published about the existence of a 7 S protein in mature oocytes, homologous to the one of previtellogenic oocytes. Using antibodies raised against the transcription factor, Honda and Roeder [7] detected no immunological reactivity with the S100 fractions of the unfertilized eggs, whereas Pelham et al. [12], using the same experimental approach, describe antibody recognition of a protein in high salt extracts from unfertilized eggs. A possible, although speculative explanation for these contradictory reports may be given assuming 2 distinct groups of major antigenic determinants on the 7 S protein in these two different antibody preparations. Thus, a putative modification of the protein extracted from mature oocytes might lead to the loss of the major antigenic determinants for one preparation of antibodies, whereas the other one

retains its activity. This putative modification might as well alter the mobility of the protein in a two-dimensional electrophoresis system [13]. This finding falls in line with the observation that in extracts from mature *Xenopus laevis* oocytes a protein with the same electrophoretic mobility as the 7 S protein derived from previtellogenic oocytes cannot be detected [13]. A modified 7 S protein might also loose part of its biological function; i.e., serving as a positive transcription factor. The relative molecular mass of the 7 S protein has been reported to be 40000–45000 [1,4,7]. Judging from its mobility in a SDS gel (fig.1B) the protein we have isolated has an $M_r$ of 45000.

Examination of the modification pattern of the free 5 S rRNA (fig.3) reveals that all the adenines modified by diethylpyrocarbonate are located in single stranded regions, except $A_{103}$, which is involved in an unstable, helix terminating A–U basepair, if we follow the general secondary structure for eukaryotic 5 S rRNA proposed by Delihas and Andersen [14]. Similar studies have been carried out on *Escherichia coli* 5 S rRNA [15] and yeast 5 S rRNA [16]. Comparison of the experimental results reveals common features such as the preferential modification of adenines in bulge loops ($A_{83}$ and $A_{49}/A_{50}$ in *Xenopus laevis*) which have been discussed to be the site of protein recognition and/or interaction [15,17]. On the other hand, there are profound differences; i.e., the accessibility of the conserved adenines in single-stranded regions E and G of *Xenopus laevis* 5 S rRNA. The structural implications of these findings will be discussed elsewhere (in preparation).

There are two effects on the modification pattern in the presence of the 7 S protein:

(1) Protection from diethylpyrocarbonate is observed in the part of the molecule comprising helices IV and V and the single-stranded regions E, F and G, with the exception of $A_{83}$ and $A_{88}$;

(2) Exposure to chemical modification of $A_{47}$ in helix III upon binding of the protein.

The protected adenines define some of the primary contact points of the 7 S protein to the 5 S rRNA and, in a first approximation, the binding region. It is noteworthy that none of the bulged adenines as putative sites of protein interaction and/or recognition is protected at its N-7 position by the bound protein. The induction of a new modification site in helix III ($A_{47}$) suggests a structural rearrangement in this part of the rRNA upon binding of the 7 S protein. Similar effects have been observed for yeast 5 S rRNA [16] when associated with ribosomal proteins, and for *Xenopus* 5 S rRNA genes it has been reported [4] that parts of the DNA become more susceptible to enzymatic cleavage when the 7 S protein, serving as a transcription factor here, is bound.

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